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# PHYSICOCHEMICAL PROPERTIES, HEAVY METALS AND MICROBIAL CONTAMINATION OF CHIA-FORTIFIED BASSISSA

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## Abstract
Microbiological and heavy metals safety are important issue for food preparation. Therefore, the objectives of the current study were investigation the physicochemical properties and microbial and heavy metals contaminations of a tradition food called bassissa. Three types of bassissa were prepared using some cereals, legumes and spices according to three different region in Libya (Gharyan, Zintan and Tripoli). Also, bassissa samples were fortified with chia powder by 5 and 10%. The obtained findings presented that Gharyan bassissa sample possessed the highest level of moisture, fat, and ash in comparison to Zintan and Tripoli samples. It was observed that chia-fortified bassissa had greater concentration of all the measured compounds compared to the control bassissa. Tripoli sample had the highest content of protein, when chia was added by 10% (24.27±0.27%); however this value did not significantly ($p \leq 0.05$) differed with that of Zintan sample (23.08±0.09%). The results of the study exhibited that chia-fortified Tripoli sample had the highest value of cadmium and cobalt. The outcomes of the studied samples displayed that the quantity of lead and tin did not detected. Tripoli sample had the highest content of Cu. The findings observed that Cu amount increased with chia addition. Tripoli sample recorded the highest value of total bacteria ($2.5\times10^4 \pm 0.01$ c.f.u/g) and the lowest was in Gharyan sample ($1.6\times10^4 \pm 0.01$ c.f.u/g). The results presented that Tripoli bassissa recorded the highest number of yeasts with or without chia powder. The same trend was observed for the number of molds, however, Zintan sample recorded the greatest number of molds.

**Keywords**: bassissa, chia, physicochemical properties, heavy metals, microbiological contamination.

## Introduction
Grains, legumes, and spices make up a large part of a person's daily diet. It is an important source of carbohydrates, protein and functional components (Bouchard et al., 2022). The products of grains such as wheat are consumed in the form of flour, semolina or a whole meal. While legumes

are consumed in the form of whole seeds or in the form of flour or powder that is added to other food formulations to form traditional meals, such as bassissa.

Bassissa is a dry food with high nutritional value and health benefits. It is prepared using cereal grains like wheat, some legumes, such as chickpea, lentils and fenugreek and spices. These ingredients are cleaned, roasted, mixed with different propositions and finally milled to fine powder. This fine powder is called bassissa, which consumed by mixing it with water or olive oil (Sayed-Ahmad et al., 2018). Chia can be added to bassissa to enhance its nutrition value and health effects, without any significant effect on the sensory properties of the final product. An investigation indicated that addition of chia to bassissa by 5 or 10% significantly enhanced its nutrition value in terms of vitamins, minerals, omega fatty acids and protein (Abu Manjil et al., 2023). It has been also indicated that chia seeds possess some antimicrobial effects against some periodontal pathogens (Divyapriya et al., 2016). A study demonstrated that burger formulated with 3 and 5% of chia seeds displayed lower count of psychrotrophic bacteria in comparison to the control samples (Zaki et al., 2018).

The increasing interest of international health organizations in recent years in traditional foods and the necessity of their consumption revealed the need to explore their nutritional, health benefits and safety. Bassissa is prepared using some grains and legumes that could be source of bacteria, mold and toxic metals contamination. Since these grains are exposed to source of these contaminants during their production. Moreover, storage conditions of these raw materials and their products, such as bassissa would cause the contamination, if these conditions were inappropriate and unsafe in terms of humidity and temperature (Khapre et al., 2020). Consuming the finished products of the grain foodstuffs (cereals, legumes, spices) might be at risk (Berghofer et al 2003). Since, it was reported that they might contain fungal and bacterial toxins. It could also contain some heavy metals that are characterized by their toxicity, such as lead, cadmium and cobalt (Eglezos, 2010).

Normally, the microbial load of cereal and its products consists of bacteria, yeasts, and fungi belonging to several different types. The activity of these microorganisms during storage and, consequently, the shelf life of the product is reliant on a variety of factors. Moisture content and water availability are the most influential parameters during storage. Accordingly, grains are typically kept at low moisture contents (12–13%) and a water activity less than 0.70. Growth of mold will occur when the moisture content in beans exceeded 18% (Kronenberg, 2022). It is assumed that extensive mixing and handling during preparation and selling could introduced some pathogens. Exposure of foods to dust or air during handling and selling is likely to elevate the number of bacteria (FDA, 2022).

Heavy metals impurities should be also taken into consideration. A study was previously conducted on the extent of contamination of wheat grain products with the amount of cadmium and lead, as the study included 11 couscous samples. The results showed that the amount of cadmium was 0.025 mg/kg and the amount of lead was 0.120 mg/kg (Filon et al., 2013). Another investigation reported that level of lead in cereal samples ranged between 0.04–0.23 mg/kg

(Ekholm et al., 2007). Cabrera et al. (2003) indicated that levels of Pb in legumes were from 0.32 to 0.70 mg/kg and nuts from 0.14 to 0.39 mg/kg in Spain. Our previous study should that chia-fortified bassissa is rich in its nutritional value and had high acceptability regarding their sensory properties. Nevertheless, its safety was not investigated; accordingly, the aims of this study were to evaluate the physicochemical, microbial and the toxic metal contaminations of chia-fortified bassissa.

## Materials and Methods

### Materials

Wheat, lentils, fenugreek, chickpeas, cumin and turmeric were collected from local markets. All the used chemicals were in the analytical grade. Potato Dextrose Agar and potato Count Agar were obtained from Oxoid. Whereas nitric and sulphuric acids were attained from Sigma.

### Methods

Bassissa was prepared with the previous ingredients, but in different proportions, based on the method of its preparation in three Libyan regions (Gharyan, Zintan, Tripoli). The method of preparation as indicated in Abu Manjil et al. (2023).

### Determination of proximate composition

Moisture, protein, ash, and fat were estimated according to the AOAC (2005) methods, while carbohydrates were calculated by difference.

### Determination of heavy metals

The amount of heavy minerals was accomplished by the graphite furnace method using atomic absorption (A6800, Japan) (USDA, 2018).

### Estimation bacteria total count

The total number of bacteria was estimated according to microbiological methods recommended by the American Public Health Association (Deibel and Swanson, 2001).

### Estimation total number of fungi

The number of yeasts and molds were estimated using potato dextrose ager made by Oxoid. The numbers of yeasts and molds were calculated according to Deibel and Swanson (2001).

### Statistical analysis

The results were statistically evaluated using the (SPSS 16, 2016), using analysis of variance (ANOVA) and using Duncan's test to find the significant differences between the means at the 5% probability level.

## Results and Discussion

### Physicochemical properties

The obtained results indicated that there are significant differences between control bassissa samples and chia-fortified bassissa samples in terms of their proximate composition (Table 1). Gharyan sample significantly recorded the highest percentage of moisture, fat, and ash compared to Zintan and Tripoli samples ($p \leq 0.05$). It can be observed that chia-fortified samples recorded greater values of the all measured components in comparison to the control bassissa. Additionally, chia-fortified samples documented higher amount of moister that can be interpreted by the fact that they had greater quantities of proteins and carbohydrates, which their capacity to bind water are high.

Tripoli sample had the highest content of protein, when chia was added by 10% (24.27±0.27); however this value did not significantly ($p \leq 0.05$) differed with that of Zintan sample **(**23.08±0.09) (Table 2). In contrast, Gharyan sample documented lower quantity of total carbohydrates, because it recorded greater values of the other components. Gharyan sample had all the raw constitutes compared to Zintan sample and greater amounts of chickpea and Fenugreek in comparison to Tripoli sample. Accordingly, most of the measured components were higher in Gharyan sample. Other studies also reported that usage of chia as an ingredients in some food formulas could enriched their nutritional value, since chai seeds are a good source of vitamins (C and B), protein, omega fatty acids and minerals (KnezHrnčič et al., 2019).

**Table 1:** Proximate composition of bassissa samples

| Component | Chia ratio | Gharyan | Zintan | Tripoli |
|---|---|---|---|---|
| Moisture (%) | %0 | $9.80±0.16^{a}$ | $6.53±0.25^{c}$ | $7.23±0.31^{b}$ |
| | %5 | $10.35±0.20^{a}$ | $7.90±0.25^{c}$ | $8.15±0.20^{b}$ |
| | %10 | $11.15±0.01^{a}$ | $8.55±0.03^{c}$ | $10.5±0.03^{b}$ |
| Ash (%) | %0 | $5.35±0.16^{a}$ | $2.64±0.03^{b}$ | $2.62±0.00^{b}$ |
| | %5 | $6.65±0.03^{a}$ | $3.49±0.07^{b}$ | $3.59±0.06^{b}$ |
| | %10 | $7.05±0.02^{a}$ | $5.15±0.01^{b}$ | $4.45±0.03^{c}$ |
| Fat (%) | %0 | $4.35±0.09^{a}$ | $2.78±0.18^{c}$ | $3.99±0.41^{b}$ |
| | %5 | $6.38±0.46^{a}$ | $4.78±0.07^{c}$ | $5.28±0.12^{b}$ |
| | %10 | $8.01±0.01^{a}$ | $5.95±0.07^{c}$ | $6.35±0.02^{b}$ |
| Protein (%) | %0 | $15.13±1.49^{b}$ | $19.08±0.29^{a}$ | $18.08±0.13^{a}$ |
| | %5 | $18.37±0.40^{b}$ | $21.65±0.24^{a}$ | $21.76±0.31^{a}$ |
| | %10 | $20.35±1.33^{b}$ | $23.08±0.09^{a}$ | $24.27±0.27^{a}$ |
| Carbohydrates (%) | %0 | $63.37±1.57^{b}$ | $68.97±0.61^{a}$ | $68.08±0.29^{a}$ |
| | %5 | $65.12±0.05^{b}$ | $70.23±0.10^{a}$ | $70.15±0.16^{a}$ |
| | %10 | $68.23±0.11^{b}$ | $70.12±1.31^{a}$ | $72.37±0.51^{a}$ |

Tabular values are mean of three replicates ± standard error. The means with similar letters in a row have no significant differences at 5% probability level.

**Heavy metals content**

Nowadays, Heavy metals contamination is a universal problem, because of the rapid development of urbanization and industrialization (Rai et al., 2019). This issue represent a considerable challenges in terms of ecological and health crisis, since their biodegradability take long time. Moreover, these metals enter the food chain via contamination of water, soil and air (França et al., 2017).

The results of the study showed that chia-fortified Tripoli sample documented the highest values of cadmium (0.045±0.04 mg/Kg) and cobalt (1.95±0.15 mg/Kg) (Table 3). In general, the results indicated that there was a significant ($p \leq 0.05$) difference between bassissa samples. It was presented that levels of Cd in different cereals (wheat, peas, corn, lentil, split peas and bean) were

0.41, 0.09, 0.29, 0.07, 0.13 and 0.15 mg/kg, respectively (Pirsaheb et al., 2016). It is stated that the acceptable level of Cd should not exceed 0.2 mg/kg (Mirlohi et al., 2017; European Community, 2006). In the current study, chia powder and chia-fortified bassissa had Cd concentration less than the critical limit.

The findings of the studied samples showed that the amount of lead and tin did not detected (Table 2). Our result is not consistent with other study that found the mean level of Pb in corn, wheat, peas, bean, lentil, and split peas were 1.5, 1.85, 1.34, 1.90, 0.95 and 1.83 mg/Kg, respectively (Naseri et al., 2015). The toxicity of Pb at the threat dose concentration can lead to an increment in blood pressure, bone weakness and nervous system problems. It might also deleteriously influence mental development, causing cardiovascular and neurological disorders in human body (Zhou et al., 2016). It is indicated that the safe level of Pb in cereal and legumes should not be higher than 0.2 mg/kg (Mirlohi et al., 2017). Accordingly, the used ingredients for basisa preparation were safe of Pb toxicity.

Tripoli sample had the highest content of Cu (3.35±0.09 mg/Kg) (Table 3). It can be observed that its amount significantly increased with chia addition except for Gharyan. Cu is an essential mineral that are required for diversity of biomolecules to maintain the ordinary structure, proliferation and function of cells (Khajeh et al., 2010). Furthermore, this metal could be toxic in excessive quantity, particularly in certain genetic illnesses (Zheng et al., 2008). An investigation found that Cu concentration in all tested cereal samples ranged between 0.55 to 6.77 mg/kg (Pirsaheb et al., 2016). Other studies established that Cu levels in cereals ranged from 1.20–3.10 mg/kg (Khajeh et al., 2010) and from 2.00– 14.00 mg /kg (Ekholm et al., 2007). The permissible level of Cu in cereal and legume foods is 73.30 mg/kg (Mirlohi et al., 2017; European Community, 2006), thus our basisa and the used chia powder had no excess Cu. It can be noted that the amount of cobalt, copper and cadmium increased with increasing chia ratio. Although, all the levels of the measured heavy metals were in the acceptable levels.

**Table 2:** Heavy metals in bassissa samples (mg/kg)

| Metal | Chia ratio | Gharyan | Zintan | Tripoli | Chia powder |
|---|---|---|---|---|---|
| **Cd** | %0 | 0.014±0.03$^{c}$ | 0.016±0.01$^{b}$ | 0.013±0.05$^{a}$ | |
| | %5 | 0.032±0.02$^{a}$ | 0.041±0.01$^{b}$ | 0.043±0.05$^{c}$ | 0.17±0.03$^{a}$ |
| | %10 | 0.038±0.03$^{a}$ | 0.043±0.06$^{b}$ | 0.045±0.04$^{c}$ | |
| **Co** | %0 | 0.99±0.00$^{c}$ | 0.81±0.03$^{a}$ | 0.95±0.10$^{b}$ | |
| | %5 | 1.03±0.00$^{a}$ | 1.05±0.00a | 1.01±0.00a | 1.25±0.01$^{a}$ |
| | %10 | 1.93±0.10$^{b}$ | 1.88±0.13$^{a}$ | 1.95±0.15$^{c}$ | |
| **Cu** | %0 | 3.10±0.01$^{a}$ | 3.13±0.01$^{a}$ | 3.35±0.09$^{a}$ | |
| | %5 | 2.10±0.01$^{a}$ | 3.12±0.01$^{b}$ | 3.56±0.01$^{b}$ | 2.14±0.02$^{a}$ |
| | %10 | 3.17±0.01$^{a}$ | 5.19±0.04$^{b}$ | 5.23±0.01$^{b}$ | |
| **Sn** | %0 | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ | |
| | %5 | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ |
| | %10 | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ | 0.00±0.06$^{a}$ | |
| **Pb** | %0 | 0.00±0.03a | 0.00±0.03a | 0.00±0.03a | 0.00±0.03$^{a}$ |

 

| | | | | |
|---|---|---|---|---|
| | %5 | 0.00±0.03[a] | 0.00±0.03[a] | 0.00±0.03[a] |
| | %10 | 0.00±0.03[a] | 0.00±0.03[a] | 0.00±0.03[a] |

Tabular values are mean of three replicates ± standard error. The means with similar letters in a row have no significant differences at 5% probability level.

**Microbial contamination**

It was observed that there was a significant decrease in the total number of bacteria for the samples of bassissa fortified with chia by 5% and 10% compared to the control bassissa except of Tripoli sample (Table 3). Tripoli sample recorded the highest value ($2.5\times10^4 \pm 0.01$ c.f.u/g) and the lowest was in Gharyan sample ($1.6\times10^4 \pm 0.01$ c.f.u/g). The International Commission for Microbiological Specifications for Foods (ICMSF, 1996) indicated that total aerobic plate counts of ready-to-eat foods should rang from zero to $10^3$. Another study also reported that total aerobic plate counts in ready to eat food were higher than the recommended limit by the International Commission for Microbiological Specifications for Foods (ICMSF, 1996). It can be seen that addition of chia powder decreased the number of total bacteria in the sample of Gharyan and Zintan. It has been showed that chia seeds possess some antimicrobial activity (Divyapriya et al., 2016).

The results presented that Tripoli bassissa recorded the highest number of yeasts with or without chia powder (Table 3). The increase in the number of yeasts in the bassissa samples was significant ($p \leq 0.05$). The same trend was observed for the number of molds, however, Zintan sample recorded the greatest (Table 3). Bassissa was prepared using some cereals, legumes and spices, accordingly the high contamination with yeast and molds could be due to the contamination of the raw ingredients. It is known that cooking temperature destroy all potential microorganisms except spore forming bacteria and thermophile (Henry et al., 2017). In this study all the ingredients were roasted before milling. The used chia powder contains higher level of yeasts and molds compared to all the control bassissa samples, this could explain the high number of molds and yeasts in the chia-fortified bassissa. A study was conducted on the contamination of chia seeds with fungi and aflatoxin in Thailand, 100 samples collected from health food stores in Bangkok. Their results indicated that ten type of molds belonging to Penicillium were isolated, and *Aspergillus Flavus* was the most prevalent among other fungi, and three samples were contaminated with aflatoxins at concentrations higher than the EU limit (4 ng/g), and aflatoxin B1 was the most dominant toxic found in chia seeds (Oyedele et al., 2020). Nevertheless, another study was performed on microbial contamination of sesame, flaxseed and chia in southern Portugal to evaluate the number of aerobic microorganisms, mold, yeast and Escherichia coli. It was found that chia recorded the lowest level $2.9\times10^4$ of all the measured microorganisms (Grancieri, 2019). The difference in the results of our study with other investigation might be related to the handling and storage condition of the used chia seeds. The results showed that the used chia seeds and the prepared basisa are unsafe in terms of microbial contamination, which might be related to the utilized raw ingredients (cereal, legumes and spices).

**Table 3:** Total number of bacteria, yeasts and molds in the studied bassissa samples (c.f.u /g)

| Microbe | Chia ratio | Chia powder | Gharyan | Zintan | Tripoli |
|---|---|---|---|---|---|
| Bacterial count test | %0 | $3\times10^3\pm0.00$ | $2.0\times10^4\pm0.01^a$ | $2.6\times10^4\pm0.01^a$ | $2.0\times10^4\pm0.01^a$ |
| | %5 | | $1.0\times10^4\pm0.01^b$ | $1.0\times10^4\pm0.01^b$ | $2.0\times10^4\pm0.01^a$ |
| | %10 | | $1.6\times10^4\pm0.01^b$ | $1.8\times10^4\pm0.01^b$ | $2.5\times10^4\pm0.01^a$ |
| Yeasts | %0 | $6\times10^3\pm0.00$ | $5.3\times10^3\pm0.55^a$ | $4.8\times10^3\pm0.33^b$ | $5.7\times10^3\pm0.20^a$ |
| | %5 | | $6.3\times10^3\pm0.27^a$ | $5.8\times10^3\pm0.20^b$ | $6.8\times10^3\pm0.52^a$ |
| | %10 | | $7.2\times10^3\pm0.81^b$ | $7.3\times10^3\pm0.75^b$ | $8.5\times10^3\pm1.00^a$ |
| Molds | %0 | $4\times10^3\pm0.01$ | $4.8\times10^3\pm0.33^b$ | $5.2\times10^3\pm0.36^a$ | $5.1\times10^3\pm0.41^a$ |
| | %5 | | $5.8\times10^3\pm0.12^a$ | $6.6\times10^3\pm0.41^a$ | $5.9\times10^3\pm0.36^a$ |
| | %10 | | $7.4\times10^3\pm0.83^b$ | $9.2\times10^3\pm1.00^a$ | $7.4\times10^3\pm0.46^b$ |

Tabular values are mean of three replicates ± standard error. The means with similar letters in a row have no significant differences at 5% probability level.

## CONCLUSIONS

The results of the current study revealed that chia-fortified bassissa samples are rich sources of fiber and protein. Moreover the prepared samples were safe of the toxic metals. However, their content of yeast and molds are exceeded the safe limits. The further studies should investigate the type of these molds and yeasts.

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**Conflict of interest**

The authors state that the work is original and has not been published before; also, it is not under consideration for publication elsewhere, all authors have read and approved the work. The authors declare that have no conflict of interest.

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